Competition for iron drives phytopathogen control by natural rhizosphere microbiomes

Shaohua Gu[1], Zhong Wei[1 ✉], Zhengying Shao[1], Ville-Petri Friman[1,2], Kehao Cao[1], Tianjie Yang[1], Jos Kramer[3,4], Xiaofang Wang[1], Mei Li[1], Xinlan Mei[1], Yangchun Xu[1 ✉], Qirong Shen[1], Rolf Kümmerli[3,4] and Alexandre Jousset[1,5]

Plant pathogenic bacteria cause high crop and economic losses to human societies[1–3]. Infections by such pathogens are challenging to control as they often arise through complex interactions between plants, pathogens and the plant microbiome[4,5]. Experimental studies of this natural ecosystem at the microbiome-wide scale are rare, and consequently we have a poor understanding of how the taxonomic and functional microbiome composition and the resulting ecological interactions affect pathogen growth and disease outbreak. Here, we combine DNA-based soil microbiome analysis with in vitro and in planta bioassays to show that competition for iron via secreted siderophore molecules is a good predictor of microbe–pathogen interactions and plant protection. We examined the ability of 2,150 individual bacterial members of 80 rhizosphere microbiomes, covering all major phylogenetic lineages, to suppress the bacterium *Ralstonia solanacearum*, a global phytopathogen capable of infecting various crops[6,7]. We found that secreted siderophores altered microbiome–pathogen interactions from complete pathogen suppression to strong facilitation. Rhizosphere microbiome members with growth-inhibitory siderophores could often suppress the pathogen in vitro as well as in natural and greenhouse soils, and protect tomato plants from infection. Conversely, rhizosphere microbiome members with growth-promotive siderophores were often inferior in competition and facilitated plant infection by the pathogen. Because siderophores are a chemically diverse group of molecules, with each siderophore type relying on a compatible receptor for iron uptake[8–12], our results suggest that pathogen-suppressive microbiome members produce siderophores that the pathogen cannot use. Our study establishes a causal mechanistic link between microbiome-level competition for iron and plant protection and opens promising avenues to use siderophore-mediated interactions as a tool for microbiome engineering and pathogen control.

Soil-borne pathogens are a global threat to food production[1–3]. Apart from the damage they inflict, a main problem is that there are very few management strategies available to control soil-borne bacterial phytopathogens. Plant root-associated microbiomes are increasingly seen as a possible driver of natural pathogen resistance and have become a target for innovative strategies aiming at improving crop protection[13–15]. However, our ability to predict and engineer microbiome function is still very limited and past research splits into two disjointed lines. The first line of studies has used comparative approaches to unravel how the microbiome structure and the prevalence of putatively pathogen-suppressive traits correlate with plant health[13,16,17]. The second line has focused on specific systems to mechanistically test whether factors such as antibiosis, resource competition and activation of plant immunity can affect plant protection[18–20]. Although the former line reveals potentially important candidate traits, species or functions involved in pathogen suppression, it provides limited insights on the underlying causal mechanisms. Conversely, the latter line offers specific mechanistic insights but it is often difficult to generalize the results beyond the specific study system. As a result, it remains largely unclear which bacterial taxa and what type of ecological interactions in the rhizosphere determine disease outcomes by soil-borne pathogens[21].

We combined the two above-mentioned lines of research to create a predictive framework applicable for disease outcomes for tomato rhizosphere microbiomes: we first taxonomically characterized 80 tomato rhizosphere microbiomes and then conducted experimental analysis using 2,150 representative bacterial members of these microbiomes. In particular, we assessed the interaction of each isolate with the pathogen *Ralstonia solanacearum* (strain QL-Rs1115)—an economically important global pathogen that causes damage to more than 200 plants species[6,7]—both in vitro and in vivo in controlled greenhouse experiments using tomato plants. We then closed the loop by using in vitro interactions as an inference tool to explain covariation in species co-occurrence under natural settings. We hypothesized that competition for iron, through the secretion of siderophores that scavenge iron from the environment, could represent a universal mechanism determining to what extent members of the soil microbiome can suppress the pathogen and protect plants. Although other mechanisms, including resource competition or antibiosis, are probably also involved in bacterial interactions, we reasoned that these mechanisms might be superseded by competition for iron due to its universal importance for bacterial growth. Our reasoning is based on recent studies indicating that siderophore-mediated competition for iron drives eco-evolutionary dynamics in natural and infectious settings[22–25]. Iron is an essential cofactor for many enzymes, yet its bioavailability is low in most soils, because iron predominantly occurs in its insoluble ferric Fe(III) form[26–28]. Many bacteria scavenge iron from the environment through the secretion of siderophores,

[1]Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, National Engineering Research Center for Organic-based Fertilizers, Nanjing Agricultural University, Nanjing, P R China. [2]Department of Biology, University of York, York, UK. [3]Department of Quantitative Biomedicine, University of Zurich, Zurich, Switzerland. [4]Department of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland. [5]Institute of Environmental Biology, Ecology and Biodiversity, Utrecht University, Utrecht, The Netherlands. ✉e-mail: weizhong@njau.edu.cn; ycxu@njau.edu.cn